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The opioid systems and the role of glial cells in the effects of opioids

Abstract

Understanding of the molecular mechanisms in the opioid systems in chronic pain should produce new, more effective methods of the pharmacotherapy of pain. Pharmacological suppression of glial activation in combination with morphine, methadone, fentanyl and buprenorphine may be an important aspect of pain therapy. Long-term use of the classical opioid analgesics in patients with chronic pain processes results in tolerance, and the search of new treatment strategies based on the recognised mechanisms of pain is an important clinical and scientific issue.

Key words: opioid peptides, opioid receptors, opioids, morphine, glia, minocycline, pentoxifylline, ibudilast

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The opioid systems

Opioid peptides

Opioid peptides are derived from three precursors: proopiomelanocortin (POMC), proenkephalin and prodynorphin. Proopiomelanocortin is the precursor of the opioid peptides α -, β - and γ -endorphin and of the non-opioid peptides ACTH, α - and β -MSH, CLIP and β -LPH. Of these peptides, the best investigated one is β -endorphin, which plays an important role in stress, transmission of nociceptive stimuli, hormonal regulation and in the regulation of immune function. Proenkephalin is the precursor of Leu- and Met-enkephalin, Met-enkephalin-Arg⁶-Gly⁷-Leu⁸, Met-enkephalin-Arg⁶-Phe⁷, BAM, peptide E and peptide F. These peptides are involved in the mechanisms of nociception, motivational processes, modulation of the extrapyramidal system and the regulation of convulsive states. Prodynorphin gives rise to dynorphin A, dynorphin B (rimorphin) and α - and β -neoendorphin. There is evidence that

certain peptides derived from prodynorphin exert non-opioid effects in addition to opioid effects and for this reason are classified as non-opioid neuropeptides [1–8]. In 1997 Zadina et al. discovered new endogenous peptides with a very high affinity and selectivity to the MOP opioid receptor. Due to their selective effect on the receptor through which morphine exerts its actions they have been called endomorphins [9]. While nothing is known about their precursors and genes, their location in the brainstem, spinal cord and nerve ganglia as well as their coexistence with the MOP opioid receptor suggest an important role in nociception [10]. They currently serve as instrumental substances in basic research [11, 12].

The opioid peptide precursors discovered so far are encoded by three genes. These genes share many structural similarities, which might indicate a shared evolutionary origin. The similarities also involve the length of peptide chains of these precursors, as proopiomelanocortin, proenkephalin and prodynorphin contain 265, 263 and 256 amino acids, respectively

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Table 1. Selected endogenous opioid peptides, their precursors and structures

Precursor	Peptide	Structure
POMC	β -endorphin ₍₁₋₃₁₎	H-Tyr-Gly-Gly-Phe-Met-Tyr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH
PENK	Met-enkephalin	H-Tyr-Gly-Gly-Phe-Met-OH
PDYN	Dynorphin A ₍₁₋₁₇₎	H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH
Unknown	Endomorphin-1	H-Tyr-Pro-Trp-Phe-NH ₂
Unknown	Endomorphin-2	H-Tyr-Pro-Phe-Phe-NH ₂

[13]. Peptides originating from these precursors have heterogenous structures and bind to different opioid receptors, but contain tyrosine as the N-terminal amino acid (Table 1).

Opioid receptors

Opioid peptides act through specific receptors. Three opioid receptors are distinguished: μ , δ and κ , currently referred to as MOP (μ -opioid peptide), DOP and KOP. Endogenous MOP receptor ligands include endomorphins, POMC-derived peptides and enkephalins. Endogenous DOP receptor ligands are enkephalins and endogenous KOP receptor ligands include prodynorphin-derived peptides [14]. The isolated receptor proteins have vary with respect to molecular mass (MOP: 65 kDa, DOP: 53 kDa, KOP: 55 and 35 kDa) [15–19]. All the three opioid receptors have been cloned (DOP [16, 17], followed by KOP [19] and most recently MOP [18]). The opioid receptors consist of seven hydrophobic transmembrane domains, three intracellular loops and the C-terminus located inside the cell, and three extracellular loops and the N-terminus located outside the cell. The opioid receptors cloned in rodents are approximately 65% homologous in terms of the amino acid sequence. The greatest similarity is found in the transmembrane domains and the intracellular loops and the greatest differences are found in the N- and C-termini and the extracellular loops. The opioid receptors in humans show a considerable similarity in amino acid sequence and in the selectivity of ligands compared to the receptors found in rodents [20–22].

Receptor cloning has made it possible to describe their molecular structure in detail, leading to significant progress in functional research. Opioid receptors are G-protein-coupled receptors. The opioid agonists of the MOP, DOP and KOP receptors inhibit adenylate cyclase via activation of G_i and G_o proteins [8, 16, 19, 23, 24]. Opioids not only affect secondary messengers but also ion-mediated signal transmission inside cells. Opioids are believed to suppress the

excitability of nerve cells by means of two mechanisms: inhibition of Ca²⁺-mediated signal transmission and augmentation of K⁺-mediated signal transmission [25]. It is difficult to understand the actions of opioids derived from proopioidmelanocortin, proenkephalin and prodynorphin as they lack specific effects on any single type of opioid receptors, MOP, DOP or KOP. The only exception are endomorphins, which are characterised by a high affinity (endomorphin-1, K_i = 360 pM; endomorphin-2; K_i = 690 pM) and a high selectivity for the MOP opioid receptor [9, 10]. Studies of opioid systems principally utilise synthetic analogues, most commonly non-peptide peptidase-resistant substances with high selectivity for specific types and often subtypes of receptors. The endogenous opioids endomorphin-1 and endomorphin-2 activate G protein similarly to the synthetic agonist DAMGO [26]. Morphine, DAMGO and endomorphin-1 activate G_{11 α} /G_{12 α} , G_{o α} and G_{13 α} proteins in a similar manner and G_{q α} /G_{11 α} and G_{s α} in a different manner, which may be the reason for the differences observed in the internalization of the MOP opioid receptor seen between morphine and these peptides [27]. It seems interesting that excision of 33 amino acid at the C-terminus does not affect the binding of DAMGO, morphine and naloxone to the MOP opioid receptor but deprives the receptor of the ability to interact with the system inhibiting the formation of cAMP by DAMGO but not by morphine. This suggests distinct differences in the possibilities to regulate the level of secondary messengers between peptide agonists, such as DAMGO, and alkaloid agonists, such as morphine [15]. Additionally it has been demonstrated in the recent years that morphine does not cause internalization of the MOP opioid receptor (shifting into the cells) and its return to the cell membrane, while DAMGO and endomorphins do [27]. This difference is currently viewed as the reason for changes in the efficacy of morphine, such as tolerance or the reduction in effectiveness in neuropathic pain.

Radioligand binding assays have demonstrated the presence of two MOP receptor subtypes in the

rat brain, subtype 1 and subtype 2, differing in terms of affinity for their selective antagonists, naloxazine and naloxazone, respectively. The presence of MOP receptor subtypes is confirmed by studies using antisense oligonucleotide which suggest that MOP receptor subtype 1, which is involved in the antinociceptive action of morphine at the higher levels of the nervous system, contains a polypeptide sequence encoded by exons 1 and 4, while the MOP receptor subtype 2, which is involved in the antinociceptive action of morphine at the level of the spinal cord and in intestinal motility, contains a polypeptide sequence encoded by exon 4 only. The presence of a third MOP receptor subtype is also postulated. The receptor would be responsible for the analgesic effects of morphine glucuronate rather than pure morphine. The amino acid sequence of this receptor is believed to be encoded by exons 2 and 3 rather than exons 1 and 4 [28]. Subtype 1 receptors show high affinity for morphine and certain enkephalins as well as synthetic DOP receptor ligands, such as DADLE (D-Ala²-D-Leu⁵-enkephalin) and DSLET (Tyr-D-Ser²-Gly-Phe-Leu⁵-Thr-enkephalin). Subtype 2 receptors, on the other hand, show low affinity for the classic MOP receptor agonists, such as morphine and DAMGO (D-Ala²-MePhe⁴-Gly-(ol)5-enkephalin) [29, 30].

Numerous studies also suggest the existence of DOP receptor subtypes, whose agonists include DPDPE and deltorphin I in the case of subtype 1 and deltorphin II in the case of subtype 2. Antagonists of subtype 1 include 7-benzylidenenaltrexone (BNTX) and D-Ala²,Leu⁵,Cys⁶-enkephalin (DALCE), while those of subtype 2 are naltriben (NTB) and naltrindole 5'-isothiocyanate (NTII) (Table 2). The existence of DOP receptor subtypes is further supported by the fact that the agonists DPDPE and deltorphin II do not exhibit cross-tolerance. Also the selective DOP antagonists DALCE and NTII differently antagonise analgesic effects of DPDPE and deltorphin II [31, 32]. Both DOP receptor subtypes may also be activated by the endogenous opioids enkephalin and β -endorphin. Studies using antisense oligodeoxynucleotides suggest that the cloned DOP receptor corresponds with subtype 2, as administration of anti-DOP-receptor antisense oligodeoxynucleotide into the lateral ventricle of the brain suppressed analgesic effects of deltorphin II but not those of DPDPE [28, 33].

Ligand binding studies also suggest the existence of KOP receptor subtypes. It is believed that specific agonists of subtype 1 include acrylaceta-
mide compounds, U69,593 and U50,488H, those of subtype 2 include bremazocine and ethylketocyclazocine and those of subtype 3 include the nalox-

one derivative NalBzoH (Table 2). There are also certain suggestions indicating the existence of subtype 4 [28].

POMC- and proenkephalin-derived endogenous opioid peptides show higher affinity for the MOP and DOP receptors than for the KOP receptor, while prodynorphin-derived peptides principally bind with the KOP receptor [34]. It should be emphasised that none of the known endogenous opioid peptides, with the exception of endomorphins, is not selective for just one opioid receptor type. Defining the roles of specific opioid receptor subtypes is of particular importance, as it is more effective to use drugs that exert their actions through various opioid receptors, such as morphine (an agonist of the MOP and DOP), methadone (an agonist of the MOP, DOP and KOP receptors), fentanyl (a potent agonist of the MOP receptor and a weak agonist of the DOK and KOP receptors), buprenorphine (a partial agonist of the MOP, DOK and KOP receptors and an agonist of the NOP [nociception peptide] receptor). The use of various types and even subtypes of opioid receptors in a rotational manner for the management of chronic pain may enable long-term and effective treatment with opioids.

Antinociceptive effects of opioids

Neuromodulation in nociceptive processes involves modulation of both the efferent and the afferent transmission of nociceptive stimuli. Peripheral neurons transmit nociceptive stimuli from nociceptors in the peripheral tissues to the dorsal cornua of the spinal cord, from where the impulses are conveyed to the hypothalamus directly through the spinothalamic tracts to the intralaminar nuclei or indirectly through the spinoreticular tracts, reticular nuclei and the periaqueductal grey matter to the ventroposterior nuclei of the thalamus, from where thalamic cells project axons to the cerebral cortex [35, 36]. The existence of inhibitory descending pathways called antinociceptive pathways is supported by the potent analgesia elicited by administration of opioids into the subarachnoid space. Also the stimulation of efferent fibres leaving the periaqueductal grey substance and reaching the posterior cornua of the spinal cord leads to potent analgesic effects [35, 36].

Immunocytochemical assays and *in situ* hybridisation have confirmed that both opioid peptides and mRNA encoding their precursors are found at all the levels of neuronal pathways. POMC-containing neurons are found in the arcuate nucleus of the thalamus, periaqueductal grey matter, thalamic nuclei, raphe nuclei, limbic system and in the nucle-

Table 2. Ligands of MOP, DOP and KOP opioid receptors and of the NOP receptor

	Type of the receptor			NOP
	MOP	DOP	KOP	
Endogenous agonists				
Endomorphin-1	X			
Endomorphin-2	X			
β -endorphin	X	X		
Met-enkephalin	x	X	x	
Leu-enkephalin	x	X	x	
Dynorphin	x	x	X	
Nociceptin				X
Synthetic agonists				
DAMGO	X			
DPDPE		X		
[D-Ala ²]deltorphan I		X		
[D-Ala ²]deltorphan II		X		
SNC80		X		
ICI 199 441			X	
ICI 174 864			X	
PD 111 7302			X	
U50 488H			X	
Bremazocine			X	
NalBzoH			X	
RO64-6198				X
RO65-6570				X
Drugs used in clinical practice				
Morphine	X	X		
Pethidine	X			
Tramadol	X	x	x	
Oxycodone	X		X	
Codeine	X			
Pentazocine			X	
Fentanyl	X	x	x	
Methadone	X	X	X	
Buprenorphine	x	x	x	X
Antagonists				
Naloxone	X	X	X	
Cyprodime	X			
CTOP	X			
Naloxonazine	X			
Naloxazone	X			
BNTX		X		
NTII			X	
NTB			X	
norBNI			X	
GNTI			X	
J-113397				X
JTC-801				X
CompB				X
NPhe [N-Phe1]-NC(1-13)NH ₂				X
Phe Ψ [Phe1 Ψ (CH ₂ -NH)Gly ₂]NC(1-13)NH ₂				X

Affinity for the receptor type: X — potent; x — weak

us of the solitary tract, from where they project to the spinal cord. Proenkephalin- and prodynorphin-containing neurons are widespread throughout the structures of the central nervous system. Large quantities of these substances are found in the periaqueductal grey substance, thalamus, raphe nuclei and in the layers of the dorsal cornua of the spinal cord. Smaller quantities are found in the cerebral cortex. Their co-localisation is common [37].

The involvement of opioid systems in the transmission of nociceptive stimuli supports the fact that electric stimulation of neurons projecting from the periaqueductal grey substance, raphe nuclei and the reticular nuclei to the spinal cord results in analgesia, which is associated with the secretion of opioids in these structures [35]. Additionally, injury to the arcuate nucleus of the hypothalamus attenuates the analgesic effects caused by electrical stimulation of the periaqueductal grey substance, where β -endorphin nerve endings are found [35]. β -Endorphin and the exogenous MOP receptor agonists, when administered into the lateral ventricle of the brain and supraspinally in rodents, show analgesic action [38, 39].

According to the anatomical data, all the three types of opioid receptors may mediate the analgesic effects of opioids. In the descending pathways, all the opioid receptor types are found in the periaqueductal grey substance, reticular nuclei of the pons (the gigantocellular and the intermediate reticular nuclei) with a predominance of MOP and KOP receptors in the raphe nuclei (the central raphe nuclei and the nucleus raphe magnus). In the ascending neuronal nociceptive pathways, in the ganglia of the dorsal radices, spinal cord and the spinal trigeminal nucleus, MOP, KOP and DOP receptors are present. In the thalamus, MOP and KOP receptors predominate with a much lower number of DOP receptors. The distribution of MOP, DOP and KOP receptors as well as their mRNAs in the spinal cord and the ganglia suggests a very important role in the modulation of nociceptive information, as reported by many authors [40–42].

In the lumbar region of the spinal cord, MOP mRNA is localised principally in layers I–II, which is a location of particular importance for nociceptive processes due to the termination of the primary C and A δ fibres and Met-enkephalin-containing fibres. This suggests a very important contribution to the modulation of nociceptive information conveyed by postsynaptic receptors localised on primary ascending fibres. MOP mRNA is also found in layers III and IV and in the ventral part of the lumbar region of the spinal cord in layers VII and VIII, which suggests

an involvement in the nociceptive impulse transmission through the spinothalamic and spinoreticular tracts. Layer IX demonstrates a very poor expression of MOP mRNA, while some expression of MOP mRNA is observed in layer X, which suggests that these receptors affect the nociceptive information conveyed to the lateral reticular nucleus, gigantocellular reticular nucleus and the lateral paragigantocellular reticular nucleus. The expression of DOP mRNA in the lumbar region of the spinal cord is relatively high with layer IX showing the highest expression, which supports the presence of DOP mRNA in motor neurons. The greatest concentration of cells which express KOP mRNA is seen in layers I and II of the lumbar region of the spinal cord. These layers also contain dynorphin-containing fibres [3, 43], which suggests that the KOP opioid receptor, similarly to the MOP receptor, plays a very important role in the modulation of nociceptive information through postsynaptic receptors on the primary ascending fibres.

In the structures of the higher levels of the central nervous system there is a strong correlation between opioid receptor mRNA and ligand binding by these receptors. At the level of the spinal cord, on the other hand, in layers I–II, the MOP, DOP and KOP receptor binding exceeds their mRNA levels, which suggests presynaptic distribution of these receptors on the endings of the primary ascending fibres reaching the spinal cord from the ganglia of the dorsal roots. On the other hand, the deeper layers of the spinal cord demonstrate a strong correlation between the opioid receptor mRNA and ligand binding with postsynaptic receptors being the most likely cause [41]. In the central nervous system, large neurons principally express DOP receptors, while intermediate and small neurons mainly express KOP receptors. Intermediate and large neurons also contain MOP mRNA. The coexistence of MOP and DOP receptors and of MOP and KOP, but not of DOP and KOP, is very likely. It is possible that MOP and DOP receptors and MOP and KOP receptors form receptor complexes which may be involved differently in the transmission of nociceptive stimuli [41]. One of the mechanisms of action of opioids involves the inhibition of neurotransmitter secretion by primary afferent fibres. *In situ* hybridisation has demonstrated opioid receptor mRNA in the ganglia of the dorsal roots [41], where bodies of the primary fibre cells are found. In the ganglia, MOP mRNA expression is very high and is observed in approximately 55% of neurons, while DOP mRNA and KOP mRNA expression amounts to 20% and approximately 18%, respectively. Undoubtedly, the opioid receptors

present in these locations play a very important role in nociceptive transmission [10].

The significance of the MOP opioid receptor in nociceptive transmission has been confirmed by studies in MOP knockout mice, which showed a lack of analgesic effects following administration of endomorphins (selective ligands of this receptor) [44]. In inflammation caused by formalin weaker effects of endomorphins than those of DAMGO and morphine are observed, while in neuropathic pain endomorphins act better than morphine [12]. Proenkephalin-derived peptides and their analogues also trigger central antinociception. In the case of endogenous peptides, this effect is brief because they are readily cleaved by proteolytic enzymes. Their analogues, on the other hand, are resistant to proteolytic enzymes and show an antinociceptive activity when administered into the ventricles and intrathecally [38, 39]. The involvement of prodynorphin-derived peptides in the mechanisms of nociception is unclear. There have been reports of the absence of analgesic effects after these peptides were administered into the lateral ventricle of the brain [4] and of the elevation of the pain threshold after intrathecal administration [6, 45]. The increased spinal dynorphin levels observed in the neuropathic pain models supports the notion that dynorphin may play an important role in chronic pain [1, 3, 5, 43], especially since both dynorphin and the KOP receptor in the spinal cord are mainly located in structures associated with transmission of nociceptive stimuli. Research has also shown that high doses of intrathecal dynorphin damage the spinal cord [46]. Studies investigating the effects of dynorphin A_{1-17} on the intracellular Ca^{2+} concentration indicate its dual modulation role. High levels of dynorphin A_{1-17} have been shown to increase intracellular Ca^{2+} levels, which is associated with the activation of both the NMDA and the KOP receptors, while low levels of dynorphin A_{1-17} have been demonstrated to suppress Ca^{2+} -mediated signal transmission [47]. By increasing intracellular Ca^{2+} levels, dynorphin seems to be capable of modulating the effects of morphine, as confirmed by studies demonstrating that the calcium antagonist nifedipine potentiates antinociceptive effects and delays the development of tolerance [48]. There is an increasing body of evidence to support the involvement of NMDA receptors in the development and maintenance of neuropathic pain. Our studies have shown that the KOP receptor antagonist norbinaltorphimine (nor BNI) and 5'-guanidinenonaltrindole (GNTI) increase neuropathic pain. KOP blockade combined with simultaneous activation of endogenous dynorphin

results in dynorphin action on NMDA receptors, contributing to the development of allodynia and hyperalgesia [3]. These studies prove that dynorphin in neuropathic pain also exerts its effects through a non-opioid mechanism leading to the development of allodynia and hyperalgesia. Furthermore, intrathecal MK-801 (a non-competitive NMDA antagonist) or coadministration of antibodies to dynorphin A_{1-13} and morphine leads to complete antinociception [3, 49, 50]. This means that the tonic activation of the NMDA receptor following peripheral nerve damage contributes to the reduced efficacy of morphine in the neuropathic pain model [49, 50], which may be of value in the search of a new target for analgesic treatments. Based on the experimental research results, morphine is currently administered in combination with ketimine in the clinical practice to achieve an improved analgesia and less pronounced adverse reactions [51].

The opioid drugs that act *via* the MOP receptor continue to be the most effective analgesics available. Their efficacy in acute severe posttraumatic and postoperative pain is unquestionable [52–54]. The use of opioids in relieving acute pain, including postoperative pain, is common and uncontroversial. Effective postoperative pain relief has been proved to reduce the incidence of complications and to shorten the duration of hospitalisation [52–55]. According to the World Health Organisation (WHO) recommendations, opioids are used in cancer pain, especially in the terminal phase [56]. In patients with severe cancer pain potent opioids are administered in combination with other drugs and provide pain relief in 75–90% of the cases [54]. Of the many potential benefits of combination analgesic pharmacotherapy the most important one is the possibility of achieving additive or synergistic effects, thanks to which each of the drugs can be given in lower doses and the incidence of adverse reactions can be reduced [53]. It is common practice to co-administer two opioid drugs with similar effects on opioid receptors, such as morphine and fentanyl. In cancer patients, for instance, fentanyl may be given via the transdermal route and immediate-release morphine may be used for the treatment of breakthrough pain [53]. The issue of combining two opioid drugs is very interesting but its complete understanding requires further studies [57, 58]. In animal experiments coadministration of morphine and methadone with other MOP agonists (oxycodone, oxymorphone, fentanyl, alfentanil or pethidine) shows additive effects, which is most likely due to the differences in MOP receptor subpopulations and in the effects of the various agonists on

this receptor [58]. While morphine remains to be the principal step 3 opioid analgesic in the WHO analgesic ladder [56], given the insufficient analgesia and/or severe adverse reactions during oral morphine treatment, there is an ongoing search of novel therapies that would reduce adverse effects and improve analgesia. One of the approaches, referred to as opioid rotation or opioid switch, allows to improve the analgesic effect and/or relieve severe adverse reactions. Opioid rotation is used in the clinical practice in cases of toxicity, insufficient pain control and severe adverse reactions in the presence of good analgesic effect. Basic research has provided evidence to support the existence of incomplete cross-tolerance to individual opioids [30, 59], which may stem from hereditary differences in the affinity and activation of specific receptors by various opioids, individual differences in the pharmacokinetics of specific opioids as well as tolerance and interaction with other drugs [60, 61]. When using opioid rotation in clinical practice one should take into account dosing problems, especially the difficulty predicting analgesic effects and adverse reactions after the switch. One of the potent opioid analgesics currently used in opioid rotation regimens is methadone, as it shows analgesic effects in patients who have become tolerant to other MOP agonists [57, 58]. Another problem is neuropathic pain due to its high severity, chronic nature and refractoriness to treatment. Backonja et al. [62] recommend combination treatment with drugs with different mechanisms of action, which may be effective in patients with neuropathic pain. The authors recommend using antidepressants, anticonvulsive drugs and topical anaesthetic agents, such as lidocaine and capsaicin, in addition to opioid drugs [53].

Nociceptin (orphanin FQ) and the NOP receptor

Attempts to clone opioid receptor subtypes have led to the discovery of a new receptor called opioid receptor-like (ORL1) by some researchers and orphan receptor by others, as no endogenous ligands were known and opioids showed no affinity. The currently accepted term is the NOP (nociceptin peptide) receptor. In 1995 an endogenous peptide

ligand of this receptor was isolated termed nociceptin/orphanin (N/OFQ) [63, 64]. The precursor of N/OFQ is the pronociceptin gene, very similar to opioid precursors but showing particularly high structural similarity to prodynorphin. N/OFQ and dynorphin A are peptides with similar structures but the former does not bind to opioid receptors due the lack of N-terminal thytrosine (Table 3). The fact that these peptides are found in various neurons and show affinity for various receptors has considerable neurophysiologic significance [65, 66].

Both N/OFQ and the NOP receptor are present in many structures of the brain, spinal cord and ganglia [63, 67]. Co-localisation of N/OFQ, NOP receptors and POMC-derived peptides has been shown in the hypothalamus and the arcuate nucleus. It has already been established that the NOP receptor is also found on the enkephalinergic neurons of the arcuate nucleus, hippocampus and the amygdala and co-localisation of N/OFQ and dynorphin has been confirmed in the substantia nigra and the arcuate nucleus [68]. Nerve fibres containing N/OFQ and opioid peptides have been discovered in the spinal cord [68]. In the ganglia of the dorsal roots, N/OFQ is found only in the small neurons located in the vicinity of neurons containing substance P and CGRP. NOP receptors, on the other hand, are found on 72% of the neurons containing substance P and 82% of the neurons containing CGRP, which suggests that N/OFQ may presynaptically modulate nociceptive transmission of afferent fibres [69].

Electrophysiologic and behavioural data indicate that intrathecal administration of N/OFQ results in analgesic action [63, 70, 71]. Despite the structural similarities the pharmacological profile of N/OFQ is in many cases opposite to opioids and it has additionally been demonstrated that N/OFQ results in the suppression of opioid effects. In acute pain, intracerebroventricular (ICV) co-administration of N/OFQ and morphine to animals results in attenuated analgesic effects of morphine [72]. The ICV route also reverses the analgesic effects of selective agonists of MOP, KOP and DOP receptors (DAMGO, U50,488H and DPDPE, respectively) [73, 74]. Based on the results of many studies it may be concluded that ICV administration of N/OFQ antagonise the analgesic effects of morphine and other opioids, while the NOP receptor antagonists Nphe and PheΨ

Table 3. A comparison of the amino acid sequence of nociceptin/orphanin FQ and dynorphin A

Nociceptin/orphanin FQ	Phe-Gly-Gly-Phe -Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg- Lys -Leu-Ala- Asn-Gln
Dynorphin A	Tyr-Gly-Gly-Phe -Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu- Lys -Trp-Asp- Asn-Gln

increase the dose-dependent analgesic effects of morphine [75, 76]. *In situ* hybridisation studies have shown that intrathecal morphine activates the nociceptive system [76]. The increased activity of this endogenous antioioid system may be the reason for the reduced effectiveness of morphine in neuropathic pain and for the rapid development of tolerance [76]. Studies in NOP knockout mice have demonstrated a slower development of morphine tolerance, which confirms the functional interaction between NOP and MOP receptors and points to the important role of NOP receptors in the mechanisms underlying the development of tolerance to morphine [77].

The results of studies investigating the nociceptive system enable the search of new, more effective drugs for the treatment of neuropathic pain [78]. Many synthetic NOP receptor ligands are used in research studies (tab. 2). In clinical practice buprenorphine, a semisynthetic opioid with partial agonistic action at MOP, KOP and DOP receptors and complete agonistic action at NOP receptors. It shows more pronounced analgesic properties than morphine in neuropathic pain. Its effects on the NOP receptor seem to play an important yet poorly understood role. A significant topic of studies investigating the treatment of neuropathic pain is the combined use of opioid receptor ligands and NOP with the view to achieving optimum analgesic effects with minimised adverse reactions.

Modulation of the effects of opioids by glial activation inhibitors

Neuropathic pain is characterised by refractoriness to analgesic drugs [38, 50, 76, 79–82] and the studies performed in the recent years have shown that activated microglial cells play an important role in its development [83–86]. The cells of the glia (astroglia, oligodendrocytes, microglia) account for 70% of the central nervous system cells [87]. Recent studies of the neuroimmune changes utilising gene expression profiling in experimental animals on the neuropathic pain model have proved that activation of the gene expression cascades is necessary for the development and maintenance of neuropathic pain [88, 89]. This points to the complexity of endogenous factors that are responsible for the initiation and regulation of neuropathic pain states. Activated microglial cells start to produce numerous proinflammatory compounds, such as cytokines (IL-1 α , IL-1 β , TNF α , IL-6), chemokines (fractalkine, MIP-1 α , MIP-1 β , MCP-1) and cytotoxic compounds (iNOS, free oxygen and nitrogen radicals)

[85, 86, 90–92]. Further processes include the induction of various surface receptors (such as TNFRI, TNFRII, IL-1RI, CX₃CR1) which accelerate immune response [90, 93]. Recent studies have demonstrated that glial inhibitors, such as propentofylline, pentoxifylline, fluorocitrate and minocycline, suppress the secretion of numerous cytokines by reducing the activation of microglia and suppressing the development of neuropathic pain [83, 93–96].

It seems interesting that the neuroimmune changes in the course of neuropathic pain development and in morphine tolerance at the molecular level seem to be similar and concern the activation of microglial cells [79, 84–86, 88, 95]. Chronic administration of morphine in neuropathic pain has been shown to additionally increase microglial proliferation contributing to the development of tolerance [84, 95, 97]. The mechanism of morphine effects on the glia is still unknown, although it has been established that morphine changes the morphology and function of the microglia increasing, for instance, the secretion of proinflammatory cytokines, substances that suppress the effects of morphine [85, 86, 92, 95, 97]. Many authors have shown that cytokines, as a result of activation by morphine, trigger changes in the activation of MAPK and PKC kinase cascades affecting, in consequence, intracellular signalling pathways [98]. For this reason a hypothesis was proposed several years ago according to which inhibition of glial activation could not only attenuate the development of neuropathic pain but also improve the effectiveness of morphine and other drugs [85, 86, 91, 95, 99].

Song and Zhao were the first to conduct studies on an animal model [99]. They showed that administration of the glial inhibitor fluorocitrate reduced the development of morphine tolerance. Further studies on animal models of neuropathic pain, including ours, have shown that propentofylline and pentoxifylline improve analgesic properties of morphine in inflammation [100, 101]. Recently, similar results have been obtained by giving pentoxifylline to patients in the clinic [102] and their studies have proved that pentoxifylline significantly reduces morphine requirement in the postoperative period in patients undergoing cholecystectomy. Reduced blood levels of TNF α and IL-6 following surgery have been observed in these patients. A recent clinical study by Lu et al. [103] has confirmed that pentoxifylline relieves postoperative pain and very beneficially improves the effectiveness of morphine as well as causing a more rapid restoration of intestinal function. The authors have also shown that these effects are associated with changes in the produc-

tion of IL-6, IL-8 and the IL-1 receptor antagonist in the postoperative period.

Our studies in mice and rats, as well as other studies, suggest that pentoxifylline and minocycline both reduce the development of neuropathic pain in mice and rats and significantly increase the effectiveness of morphine on a neuropathic pain model [83, 84, 104]. Chronic administration of morphine in neuropathic animals results to complete development of tolerance, while glial inhibitors delay it [84, 97]. The results of western blot and immunohistochemistry indicate that minocycline and pentoxifylline considerably delay the development of morphine tolerance by reducing the degree of microglial activation as a result of chronic administration of morphine [84, 97]. Minocycline, which readily passes the blood-brain barrier, seems to be a promising substance in the treatment of neuropathic pain. It is already being used in the clinical practice for the treatment of Parkinson's disease and shows neuroprotective properties, although there are no clinical data on using it in neuropathic pain.

Studies by Ledebøer et al. [105] on animal models of neuropathic pain have shown that ibudilast (AV411), a non-selective phosphodiesterase inhibitor, suppresses the activation of glial cells, elevates the IL-10 concentration, reduces the levels of IL-1 β , TNF α and IL-6 and increases the effectiveness of morphine. Preclinical studies are ongoing in Australia at the moment, and their studies confirm that ibudilast readily passes the blood-brain barrier, is well tolerated, may be used orally, reduces glial activation, relieves the symptoms of neuropathic pain and increases morphine analgesia [106].

Understanding of the molecular mechanisms in the opioid systems in chronic pain should produce new, more effective methods of the pharmacotherapy of pain. Pharmacological suppression of glial activation in combination with morphine, methadone, fentanyl and buprenorphine may be an important aspect of pain therapy. Long-term use of the classical opioid analgesics in patients with chronic pain processes results in tolerance, and the search of new treatment strategies based on the recognised mechanisms of pain is an important clinical and scientific issue.

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